Conditioning taste aversions to locoweed (Oxytropis sericea) in horses

J. A. Pfister, B. L. Stegelmeier, C. D. Cheney, M. H. Ralphs and D. R. Gardner

J Anim Sci 2002. 80:79-83.

Conditioning taste aversions to locoweed (*Oxytropis sericea*) in horses[1]

J. A. Pfister*[2], B. L. Stegelmeier*, C. D. Cheney†, M. H. Ralphs*, and D. R. Gardner*

*USDA-ARS Poisonous Plant Research Laboratory, Logan, UT 84341 and †Department of Psychology, Utah State University, Logan, 84322

ABSTRACT: Locoweed (*Oxytropis sericea*) is a serious poisoning problem for horses grazing on infested rangelands in the western United States. Our objectives were to determine 1) whether lithium chloride or apomorphine would condition aversions to palatable foods, and at what doses, and 2) whether horses could be averted to fresh locoweed in a pen and grazing situation. Apomorphine was not an acceptable aversive agent because at the dose required to condition an aversion (≥0.17 mg/kg BW), apomorphine induced unacceptable behavioral effects. Lithium chloride given via stomach tube at 190 mg/kg BW conditioned strong and persistent aversions to palatable feeds with minor signs of distress. Pen and grazing tests were conducted in Colorado to determine if horses could be averted to fresh locoweed. Pen tests indicated that most horses (5/6) were completely averted from locoweed. Treated horses ate 34 g of fresh locoweed compared to 135 g for controls ($P < 0.01$) during three pen tests when offered 150 g per test. One horse (T) in the treatment group ate locoweed each time it was offered in the pen, but ate no locoweed while grazing. In the grazing trial, control horses averaged 8.6% of bites of locoweed ($P < 0.01$) during the grazing portion of the study, whereas treated horses averaged <0.5%. One treated horse (S) accounted for all consumption; he consumed 15% of his bites as locoweed in a grazing bout on d 2 of the field study. Thereafter, he was dosed a second time with lithium chloride and ate no locoweed in the subsequent 5 d. Three of six horses required two pairings of lithium chloride with fresh locoweed to condition a complete aversion. The results of this study indicate that horses can be averted from locoweed using lithium chloride as an aversive agent, and this may provide a management tool to reduce the risk of intoxication for horses grazing locoweed-infested rangeland.

Key Words: Food Preferences, Poisonous Plants

 J. Anim. Sci. 2002. 80:79–83

Introduction

Locoweed (*Oxytropis and Astragalus* spp.) is the most widespread poisonous plant problem in the western United States (James et al., 1981). Locoweed contains the indolizidine alkaloid swainsonine, which inhibits lysosomal α-mannosidase and mannosidase II (Dorling et al., 1980). Inhibition of these enzymes results in oligosaccharide accumulation in lysosomes with characteristic vacuolated cells (Stegelmeier et al., 1995). Clinically, locoweed disease is characterized by depression, proprioceptive deficits, intention tremors, nervousness, emaciation, and death (James et al., 1970).

Whitepoint locoweed (*Oxytropis sericea*) is widely distributed throughout the western United States, and is reported to be palatable to horses (Marsh, 1909), cattle (Ralphs et al., 1987), and sheep (Marsh, 1909). Horses appear to be especially susceptible to locoweed intoxication, and overt signs of intoxication may be more apparent in horses compared to other animals (Marsh, 1909, Stegelmeier, unpublished data). Anecdotal observations suggest that some horses often seek out locoweed in preference to other forages (Marsh, 1909; L. F. James, personal observations). Houpt et al. (1990) showed that horses can be trained to avoid foods using conditioned aversions. Cattle have been trained to avoid locoweed through conditioned taste aversions (Ralphs et al., 1997). Such an approach may provide a management tool for horses grazing in locoweed-infested pastures (Ralphs and Provenza, 1999). The objectives of this study were to determine: 1) the appropriate drug and dose to condition a complete aversion to palatable foods and 2) if horses could be averted from locoweed (*O. sericea*) in a pen and field-grazing setting.

[1]We thank Kermit Price, Ed Knoppel, Al Macilius, Rex Probst, and Danny Hansen for excellent technical assistance. We thank Fred Provenza and Beth Burritt for helpful discussions and comments. We also thank Tony Knight, Gary Greathouse, and Joel Vaad for their assistance and for allowing field research at the Colorado State University Research Foundation, Maxwell Ranch, Livermore, Colorado.

[2]Correspondence: 1150 E. 1400 N. (phone: 435-752-2941; fax: 435-752-5681; E-mail: jpfister@cc.usu.edu).

Received April 26, 2001.

Accepted August 15, 2001.

Materials and Methods

Determining the Aversive Agent and Dose

Preliminary Dose-Response Trial. We investigated the use of lithium chloride (LiCl) and apomorphine hydrochloride as aversive agents to condition an aversion. Houpt et al. (1990) used apomorphine injections at 0.067 mg/kg BW to condition partial aversions in horses to various feeds. Because apomorphine at 0.067 mg/kg did not condition complete aversions (total abstinence) in the study of Houpt et al. (1990) with horses, we determined the dose of apomorphine that would condition a complete aversion in horses. Lithium chloride has been the primary drug of choice in laboratory animals and livestock (Ralphs and Stegelmeier, 1998, Ralphs and Provenza, 1999); thus we also determined if LiCl was suitable for use as an aversive agent in horses. All protocols were approved by the Utah State University Institutional Animal and Use Committee, and were conducted under veterinary supervision in Logan, Utah. The daily basal diet for all horses in all trials was 5 to 6 kg of good quality grass hay fed at either 0900 or 1400. For all tests, horses were fasted overnight and did not receive feed at 1400.

In the preliminary assessment, two subjects were given increasing doses of LiCl and apomorphine (one subject with each aversive agent) to determine an effective dose and to observe any adverse reactions. After an overnight (18 h) fast, two horses (372 kg BW) were offered 200 g of the novel food calf manna, then immediately dosed with either LiCl at 30 mg/kg BW in 1,000 mL of tap water via oral (nasal) gavage (i.e., stomach tube), or apomorphine given by i.m. injection at 0.05 mg/kg BW in a volume of 5 mL of sterile saline. Horses were carefully monitored visually and with a stethoscope for signs of stress including heart rate, respiration, and gastrointestinal motility. The horses were tested individually for an aversion 2 d after the pairing by offering 250 g of the novel feed for 5 min. Both horses ate all the offered calf manna after the initial dose of aversive agent. We continued this dose-response test on a twice-weekly basis with these two animals, gradually increasing the dose of LiCl by 20 mg/kg (i.e., 50, 70, 90, 110, 130 mg/kg) and increasing the dose of apomorphine by 0.02 mg/kg each pairing (i.e., 0.07, 0.09, 0.11, 0.13, 0.15, 0.17 mg/kg). The LiCl subject showed some signs of rejecting the calf manna at doses equal to or greater than 90 mg/kg, and was totally averted to the calf manna at 130 mg/kg (i.e., sixth exposure). Because the apomorphine subject showed no signs of rejecting the calf manna at 0.11 mg/kg, and the calf manna was becoming more familiar (less novel) with each exposure, the apomorphine subject was exposed to another novel food (milo) for three subsequent apomorphine doses, and finally averted to milo with apomorphine at 0.17 mg/kg BW. Novel flavors provide stronger associations with nausea and thus condition stronger aversions (Garcia et al., 1985).

Aversive Agent and Dose Trial. The purpose of this trial was to test the dose of LiCl and apomorphine as determined in the preliminary trial. The trial was conducted in Logan, Utah. Six horses were dosed by stomach tube with LiCl paired with calf manna, four were dosed with apomorphine injections paired with milo, and two horses were controls. All horses were offered 250 g of the test (i.e., novel) food on d 1 after an overnight (18 h) fast, dosed with the aversive agent, then tested on d 3, 7, 14, and 30 to determine the strength and persistence of the aversion. We began dosing with LiCl at 150 mg/kg BW and apomorphine at 0.17 mg/kg BW. We selected a slightly higher initial LiCl dose because the subject in the preliminary trial showed no signs of gastrointestinal malaise (e.g., heart rate, respiration) at 130 mg/kg. The tests were conducted after an 18-h fast, and horses were given 5 min to eat 250 g of the test feed. If any horses ate any of the novel feed during the test on d 3, they were given an increased dose (LiCl: + 20 mg/kg; apomorphine: + 0.02 mg/kg). No horse required more than two doses of either aversive agent; thus, the highest doses given were 190 mg/kg of LiCl and 0.19 mg/kg of apomorphine. Controls were given five doses (one each week) by stomach tube of saline solution (equivalent volume as LiCl) paired with calf manna, and five saline injections i.m. (equivalent volume as apomorphine) paired with milo.

*Averting Horses to Locoweed (*O. sericea*)*

Conditioning Horses to Eat Fresh Locoweed. Horses (n = 10; 435 kg BW) were randomly assigned to either a treatment or control group. Horses were transported to the Colorado State University Research Foundation Maxwell Ranch near Livermore, Colorado and penned in a portable corral (N40° 56.22' W105° 15.71') in a locoweed (*O. sericea*)-infested pasture. Most locoweed plants were in the bud and flower stages of growth; none was available in the corral. After an overnight (18 h) fast, all horses were offered freshly-harvested locoweed for 10 min. All horses refused the plant on d 1. Over the next 3 d, horses were conditioned to eat locoweed by mixing fresh locoweed with clipped green grass (initially 50:50 by weight) twice daily and then were offered freshly-harvested locoweed alone.

Averting Horses to Locoweed. Once all horses were eating locoweed readily, each horse was offered 150 g (fresh weight) of locoweed for 5 min on d 6 at 0715. All horses ate locoweed ($\bar{x}$ = 110 ± 28 g) and were dosed immediately thereafter beginning at 0730. Horses in the treated group (n = 6) were gavaged with LiCl at 190 mg/kg BW in 1000 mL tap water, and controls (n = 4) were gavaged with an equivalent volume of saline (0.9% NaCl). Each horse was fed 3 kg of grass hay at 1500 on d 6 (one-half of the normal daily allotment). All horses ate readily and were then without food overnight before the d 7 test. Horses were each fed 6 kg of grass hay at about 1600 daily except on d 8 and 13 when they were given one-half that amount. Water and trace

mineral block were provided ad libitum. Horses were tested in a pen setting for aversions by offering locoweed individually to all horses on d 7, 9, and 14. For each test, horses were kept off feed overnight, and 150 g of freshly harvested locoweed was offered for 5 min at 0800.

Grazing Tests of Locoweed Consumption. The grazing trial was conducted during d 7 to 13. The grazing test was conducted simultaneously with the pen aversion study. To determine the strength of the aversion in a grazing setting, horses were individually staked on a 10-m tether (i.e., 314 m^2 grazing circle) twice daily for 10 min each time in a predetermined random order in a locoweed-infested area. The center point was offset every other day to minimize trampling damage. Bite counts were used to determine horses' diets (Pfister et al., 1997a); bites were categorized as locoweed (whole plant, flower, dried pods, or leaves), grasses, and other forbs. Two experienced observers counted bites using handheld tally counters. No attempt was made to document interobserver accuracy, but other grazing trials (e.g., Pfister et al., 1997b) have shown close agreement between these observers. Dominant grasses were needle-and-thread (*Stipa comata*), squirreltail (*Elymus elymoides*), and blue grama (*Bouteloua gracilis*).

Locoweed plant density was determined by systematically placing 16, 0.5 m^2 quadrats within each grazing circle and counting all erect locoweed plants in each quadrat. Available grass, forb, and locoweed were estimated every other day by clipping two randomly placed, 0.25-m^2 quadrats to ground level, and drying and weighing the clipped material.

Statistical Analysis

No statistical analysis was done on the dose-response trial because the 190 mg/kg dose of LiCl conditioned complete aversions (i.e., no consumption of target food). Consumption of locoweed in pen and grazing trials was analyzed using the GLM procedure of SAS (SAS Inst., Cary, NC) with repeated measures. The model included treatments (i.e., averted vs controls), subjects nested with treatments, days and day × treatment interaction. The error term for comparing treatments was the animal-within-group interaction, and the day x animal-within-group interaction was used to test day and day × treatment effects.

Results

Apomorphine

Apomorphine did not condition aversions at doses below 0.17 mg/kg as treated horses ate all the offered feed. We initially dosed four horses with apomorphine at 0.17 mg/kg, and they all exhibited marked nervousness and hyperactivity. Two apomorphine-treated (0.17 mg/kg) horses that ate the novel feed on d 3 were injected with apomorphine at 0.19 mg/kg. These two subjects were averted to the novel feed. We subsequently discontinued further testing of apomorphine because the drug induced unacceptable behavioral effects (i.e., extreme nervousness and excitability).

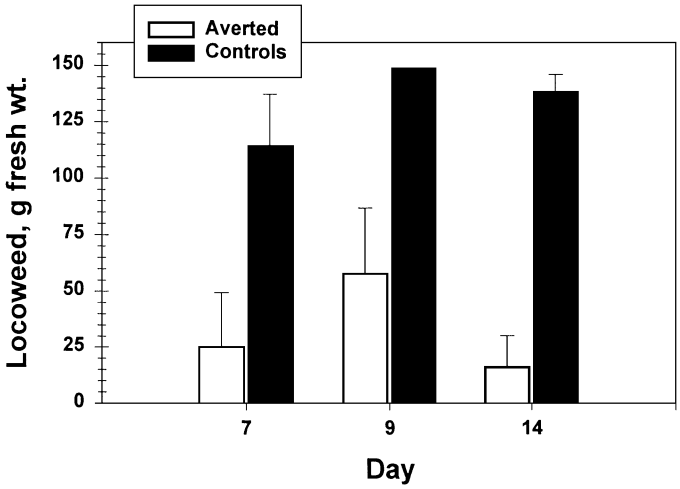

Figure 1. Consumption of locoweed (g fresh wt. ± SE) by control (solid bars) and averted (open bar) horses ($P < 0.01$) in three pen trials during a 14-d study. Horses were averted on d 6 and offered 150 g for each subsequent test.

LiCl-Dose Response to Palatable Feeds

Doses of LiCl of 190 mg/kg were completely effective in conditioning aversions to either milo or calf manna, as none of the treated horses (n = 6) ate any calf manna or milo in subsequent tests. Controls (n = 2) ate all of the offered feed (i.e., calf manna or milo) in every trial, indicating that passing the stomach tube via the nostrils was not aversive.

Aversion to Locoweed in Pen

Horses were naive to locoweed so they had to be conditioned to eat locoweed at the beginning of the study. On d 1 all horses refused to eat freshly harvested locoweed. For the next 2 d, they were given locoweed mixed with fresh green grass twice daily. On d 4, all horses ate locoweed when offered 600 g of fresh plant ($\bar{x}$ = 395 ± 56 g).

Pen tests indicated that most, but not all horses (5/6), were completely averted from locoweed (Figure 1). Averted horses ate 34 g of fresh locoweed compared to 135 g for controls ($P < 0.01$). There was no day effect nor day × treatment interaction ($P > 0.05$). One averted horse (T) ate locoweed each time it was offered in the pen test, and his consumption accounted for most of the group's consumption. Two other averted horses (S and O) ate about 50 g of locoweed on d 9. Those two horses were given a second dose of LiCl just before going out to graze in the morning; both horses ate no locoweed in the next pen trial on d 14.

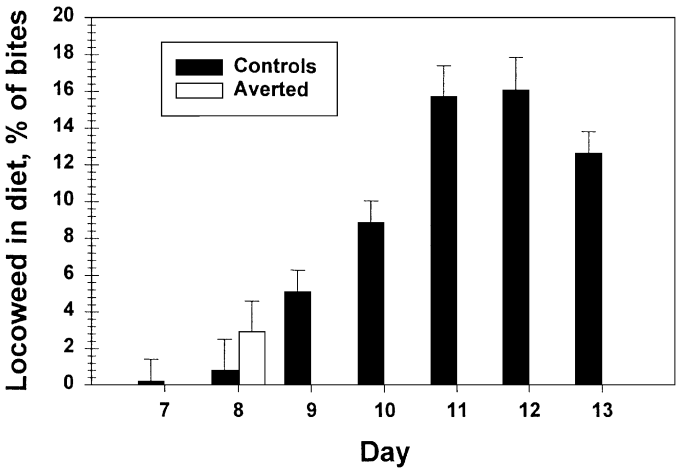

Figure 2. Consumption (% of bites ± SE) of locoweed by averted (open bar) and control (solid bar) horses during a 7-d grazing trial in Colorado. All consumption on d 8 for the averted group (n = 6) was by one horse.

Aversion to Locoweed When Grazing

Locoweed density (i.e., erect plants) in the grazing area was 9.5 and 4.5 plants/m^2 on d 7 and 12, respectively. Grazing circles averaged 273 ± 29 and 140 ± 41 kg/ha of grasses and locoweed, respectively. Production of all forage plants, including locoweed, was lower than normal due to spring drought (J. Vaad, personal communication).

Control horses averaged 8.6% of bites as locoweed during the grazing portion of the study, whereas treated horses averaged <0.5% ($P < 0.01$) (Figure 2). Horse S accounted for all the locoweed consumption by treated horses. On d 8 (second day of grazing trial), this horse ate 15% of his bites as locoweed during the morning grazing period. This was the only instance of locoweed consumption by a treated horse during the 7-d grazing study. Interestingly, horse T ate harvested locoweed during pen tests, but did not eat locoweed while grazing.

Discussion

The toxic dose of locoweed to horses has not been specifically determined, but horses seem to be more sensitive to the locoweed toxin, swainsonine, than are other livestock species (Marsh 1909; Stegelmeier, unpublished data). Sheep show signs of chronic intoxication when they ingest 0.21 mg of swainsonine·kg^{-1}·day^{-1} for several weeks (Pfister et al. 1996).

Houpt et al. (1990) reported that apomorphine-conditioned aversions in horses at doses of 0.067 mg/kg BW, and Zahorik et al. (1990) found that apomorphine induced aversions in cattle, sheep, and goats with doses of 0.1 mg/kg. We found, however, no evidence of aversions with apomorphine doses at or below 0.1 mg/kg. We noted partial aversions at doses at or above 0.17 mg/kg. Ralphs and Stegelmeier (1998) noted that apomorphine conditioned partial aversions (dose of 0.2 mg/kg) to a novel feed in cattle, and those transient aversions extinguished within 6 d during further tests. Higher doses (e.g., 0.4 mg/kg) were debilitating and sometimes fatal (Ralphs and Stegelmeier, 1998).

It is not possible to directly compare apomorphine and LiCl. Apomorphine cannot be administered orally because the drug is extensively conjugated by the liver before it reaches the systemic circulation (Smith et al., 1981), whereas LiCl is typically given in a diluted solution via gastric lavage due to its hygroscopic and caustic properties. Apomorphine is reported to have a short duration of action (Goldberg et al., 1981), and thus may not condition strong food aversions compared to a slower acting aversive agent like LiCl (Johnson et al., 1980; Ralphs and Stegelmeier, 1998). Ralphs and Stegelmeier (1998) reported that apomorphine-treated cattle were ill within 10 min of injection, but recovered quickly from the apomorphine-induced illness. In contrast, LiCl-induced nausea may be effective for many hours post-dosing (Ralphs and Stegelmeier, 1998). In addition, apomorphine may produce weaker aversions because the drug apparently works centrally in the CNS rather than causing gastrointestinal nausea (Pratt and Stolerman, 1984). Although apomorphine is less stable in solution than LiCl, intramuscular injections with apomorphine provide an easier dosing route for an aversive agent than dosing LiCl by stomach tube. Nonetheless, apomorphine is not suitable for use as an aversive agent in horses because the dose required to form an aversion provoked undesirable behavioral responses. Treated horses were uncontrollable, often rearing and appearing to lose some proprioceptive abilities, while trying to jump any obstacle (e.g., fences, gates) in their path. Similar rearing behaviors have been induced by high apomorphine doses in rats (Antoniou and Kafetzopoulos, 1991). Furthermore, apomorphine did not condition complete aversions in all animals even at high doses (i.e., ≥0.17 mg/kg BW).

These results indicate that the aversions conditioned using LiCl were strong and persistent, given that we used palatable foods, the horses were fasted overnight before being tested, and avoidance persisted at least 30 d. Ralphs and Provenza (1999) noted that LiCl-induced aversions can last for >3 yr in cattle with no subsequent treatment. In our trials, the averted horses initially smelled the offered food because they were hungry, and then backed away and did not approach the container during the remainder of the test. Houpt et al. (1990) suggested that it might be more difficult to avert horses to palatable foods like corn, but in our study, once we arrived at the proper aversive agent and dose, horses easily formed aversions to palatable foods such as calf manna.

The 190 mg/kg dose of LiCl caused no overt signs of gastrointestinal distress or colic. Doses of LiCl lower than 190 were inconsistent in conditioning an aversion. Apparently LiCl has milder effects on horses than on

cattle at similar doses (200 mg/kg BW; Ralphs and Stegelmeier, 1998), as we observed no inappetence and only mild diarrhea in a few horses the day after dosing. We dosed one horse with LiCl in incremental doses up to 400 mg/kg BW under veterinary supervision, and this animal showed only mild overt signs of gastrointestinal malaise, suggesting that the safety margin for use of LiCl in horses is broad. Johnson et al. (1980) reported that cattle showed signs of intoxication when given LiCl at 250 mg/kg, and doses above 500 mg/kg were fatal to cattle.

Implications

Cattle have been conditioned to avoid locoweed and other toxic plants under grazing situations. The results of this study indicate that horses may be averted from locoweed (*Oxytropis sericea*) using lithium chloride as an aversive agent at 190 mg/kg BW. Our results also suggest that naive horses can quickly learn to accept locoweed and that some horses will require more than one pairing of the aversive agent with the taste of locoweed in order to condition an aversion. Although it seems that most horses will avert from locoweed, there may be some individual horses that will not avert even with repeated doses of lithium chloride. Further work is needed to determine 1) whether experienced horses can be averted from locoweed when actively grazing the plant, 2) whether horses can be averted from other palatable and intoxicating locoweed species such as *Astragalus lentiginosus* (specklepod locoweed), and 3) whether aversions will persist for years without further doses of aversive agent.

Literature Cited

Antoniou, K., and E. Kafetzopoulos. 1991. A comparative study of the behavioral effects of d-amphetamine and apomorphine in the rat. Pharmacol. Biochem. Behav. 39:61–70.

Dorling, P. R., C. R. Huxtable, and S. M. Colegate. 1980. Inhibition of lysosomal α-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. Biochem J. 191:649–651.

Garcia, J., P. S. Lasiter, F. Bermudez-Rattoni, and D. A. Deems. 1985. A general theory of aversion learning. Ann. N. Y. Acad. Sci. 443:3–41.

Goldberg, D. M., G. D. D'Mello, S. R. Goldberg, and I. P. Stolerman. 1981. Conditioned taste aversion and operant behavior in rats: Effects of cocaine, apomorphine, and some long-acting derivatives. Pharmacol. Exp. Ther. 219:60–68.

Houpt, K. A., D. M. Zahorik, and J. A. Swartzman-Andert. 1990. Taste aversion learning in horses. J. Anim. Sci. 68:2340–2344.

James, L. F., W. J. Hartley, and K. R. Van Kampen. 1981. Syndromes of *Astragalus* poisoning in livestock. J. Am. Vet. Med. Assoc. 178:146-150.

James, L. F., K. R. Van Kampen, and A. E. Johnson. 1970. Physiopathologic changes in locoweed poisoning in livestock. Am. J. Vet. Res. 31:663–672.

Johnson, J. H., H. R. Crookshank, and H. E. Smalley. 1980. Lithium toxicity in cattle. Vet. Hum. Toxicol. 22:248–251.

Marsh, C. D. 1909. The loco-weed disease of the plains. USDA Bureau of Anim. Ind. Bull. 112, Washington, D.C.

Pfister, J. A., F. D. Provenza, G. D. Manners, D. R. Gardner, and M. H. Ralphs. 1997a. Tall larkspur ingestion: Can cattle regulate intake below toxic levels? J. Chem. Ecol. 23:759–777.

Pfister, J. A., M. H. Ralphs, G. D.Manners, D. R. Gardner, K. W. Price, and L. F. James. 1997b. Early season grazing by cattle of tall larkspur-(*Delphinium* spp.) infested rangeland. J. Range Manage. 50:391–398.

Pfister, J. A., B. L. Stegelmeier, C. D. Cheney, L. F. James, and R. J. Molyneux. 1996. Operant analysis of chronic locoweed intoxication in sheep. J. Anim. Sci. 74:2622–2632.

Pratt, J. A., and I. P. Stolerman. 1984. Pharmacologically specific pretreatment effects on apomorphine-mediated conditioned taste aversions in rats. Pharmacol. Biochem. Behav. 20:507–511.

Ralphs, M. H., D. Graham, M. L. Galyean, and L. F. James. 1997. Creating aversions to locoweed in naive and familiar cattle. J. Range Manage. 50:361–366.

Ralphs, M. H., L. V. Mickelsen, and D. L. Turner. 1987. Cattle grazing white locoweed: Diet selection patterns of native and introduced cattle. J. Range Manage. 40:333–335.

Ralphs, M. H., and F. D. Provenza. 1999. Conditioned food aversions: principles and practices, with special reference to social facilitation. Proc. Nutr. Soc. 58:813–820.

Ralphs, M. H., and B. L. Stegelmeier. 1998. Ability of apomorphine and lithium chloride to create food aversions in cattle. Appl. Anim. Behav. Sci. 56:129–137.

Smith, R. V., A. E. Klein, R. E. Wilcox, and W. H. Riffee. 1981. Apomorphine: bioavailability and effect on stereotyped cage climbing in mice. J. Pharm. Sci. 70:1144–1146.

Stegelmeier, B. L., R. J. Molyneux, A. D. Elbein, and L. F. James. 1995. The lesions of locoweed (*Astragalus mollissimus*), swainsonine, and castanospermine in rats. Vet. Pathol. 32:289– 298.

Zahorik, D. M., K. A. Houpt, and J. Swartzman-Andert. 1990. Taste aversion learning in three species of ruminants. Appl. Anim. Behav. Sci. 26:27–39.